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A Bacteriologic Study of Human Dental Periapical Abscesses

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A BACTERIOLOGIC STUDY OF HUMAN DENTAL PERIAPICAL ABSCESSES

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INTRODUCTION

THE PROBLEM

A dental periapical abscess is a localized collection of pus in the alveolar bone of the apex of a tooth. Various terms have been used to describe this pathologic entity in the dental literature. These include: acute alveolar abscess (Gilmer and Moody, 1914), apical abscess (Head and Ross, 1919), periapical abscess (Fraser, 1923), acute dentoalveolar abscess (Alin and Ågren, 1954), and submucous abscess (Feldmann and Larje, 1966). Many cases of such abscesses apparently present annually throughout the world. Whereas, there are no published data confirming the actual incidence of these lesions, an examination of the dental emergency records of the University of Connecticut Health Center at Farmington, Connecticut for 1980 revealed 324 cases of dental abscesses out of a total of 4,697 patients seen. This figure represents approximately 7.5% of all the patients seen and underscores in this instance the high frequency of occurrence of these lesions. These commonly painful abscesses if left untreated, may proceed to even more severe sequelae. Some of the sequelae that have been reported include obstruction of the airway (Cogan, 1973; Linkous and Welch, 1975), brain abscesses (Gold, 1949; Hollin et. al., 1967; Tatoian et. al., 1975), fatal mediastinitis (Cogan, 1973), erosion of major

vessels with concommitant severe hemmorrhage (Linkous and Welch, 1975) and loss of various functions, for example, blindness, (Gold and Sager, 1974). The incidence of such dental periapical abscesses has been associated with frank dental carious lesions with attendant pulpal exposure or with concommitant periodontal lesions. In addition, they have also been associated with traumatized teeth with pulpal necrosis (Sundqvist, 1976). Sometimes, however, a small percentage of such abscesses present with no apparent antecedent pathology. It is known that microbial infection of the necrotic pulp tissue results in inflammation of the periapical tissue (Bergenholtz, 1974; Block et. al., 1976; Sundqvist, 1976). Whereas such inflammatory reactions are often of an asymptomatic nature, they are sometimes painful when the inflammation is accompanied by rapid tissue destruction and subsequent abscess formation (Block et. al., 1974; Grossman, 1978; Shklar, 1979). Currently, controversy exists concerning the specific etiology of such abscesses.

REVIEW OF THE LITERATURE

THEORETICAL ASPECTS

Earlier histologic studies of chronic dental periapical lesions reported variable findings. In some studies, the presence of bacteria was frequently noted (Boyle, 1934; Winkler et. al., 1972), while in others they appeared to be consistently absent (Harndt, 1926; Andreasen and Rud, 1972; Langeland and Block, 1977). The finding of bacteria within periapical lesions such as the granuloma by some of these earlier workers is probably related to inadequate methodologies (Langeland, 1977). Whereas more direct microbiological studies have been performed to describe the microflora of necrotic dental pulps there have been but a few limited *in situ* investigations of the possible microbial association with dental periapical abscesses (Alin and Ågren, 1954; Feldmann and Larje, 1966). There are several reports of the bacteriological status of periapical lesions from teeth subsequent to their extraction (Mela, 1934; Gier and Mitchel, 1968; Melville and Birch, 1967). However, data derived from such studies should be considered with some caution because of the possibility of contamination from pulpal or periodontal sources attendant on the extraction procedure (Moller, 1966). In the few *in situ* studies thus far reported, the microflora was ascertained to be comprised

predominantly of obligate rod-shaped anaerobes and facultative anaerobic cocci (Feldmann and Larje, 1966; Sabiston, Grigsby, and Segerstrom, 1976). Unfortunately, however, in those particular investigations, there was no attempt made to clearly differentiate between abscesses of periodontal or pulpal origin. This distinction is extremely important since the microflora of periodontal pockets and necrotic dental pulps from which the abscesses may originate have been shown to differ significantly (Tanner et. al., 1979; Sundqvist, 1976). A summary of previous bacteriologic studies of dental abscesses is shown in Table I.

PROCEDURAL ASPECTS

In microbiological studies involving dental periapical abscesses, the investigator is initially faced with a number of methodolical problems such as specimen contamination by non-involved oral flora, loss of certain microorganisms during their transport, various handling procedures and an inability to culture certain fastidious microorganisms due to inappropriate atmospheric conditions and culture media used. These problems will now be considered in further detail.

a. Mucosal Surface Disinfection and Specimen Collection

Since one may anticipate that dental periapical abscesses are induced by microorganisms that are part of the normal oral microflora, it is therefore extremely important to disinfect the mucosal surface overlying the abscess, since it may well be colonized by such oral microorganisms, before

sample collection. This would ensure that samples from the abscesses do not contain extraneous contaminants from the indigenous oral flora. Thus, a variety of sampling techniques have been used in past studies.

Several workers have obtained their samples by means of swabs and/or needle aspiration, subsequent to an incision of the abscessed site which had been previously disinfected with some medicament (Gilmer and Moody, 1914; Alin and Ågren, 1954; Melville and Birch, 1967; Feldmann and Larje, 1966; Turner, Moore, and Shaw, 1975). Initially alcohol was used for surface disinfection (Gilmer and Moody, 1914), then Benzalkonium chloride and thermocauttery (Alin and Ågren, 1954), and later iodine tinctures (Melville and Birch, 1967; Turner, Moore, and Shaw, 1975). Hedman (1951) obtained samples via the root canal by means of a cannular and a culture wire, after the isolation of the involved tooth with a rubber dam and prior surface disinfection with iodine. Syringe and needle aspiration techniques as a sole method of sample collection have also been employed (Bulleid, 1931; Sabiston and Gold, 1974). In a more recent study (Sabiston, Grigsby, and Segerstrom, 1976), samples were collected by syringe/needle aspiration in some cases while sterile paper points were used in others. Bulleid (1931) utilized alcohol for surface disinfection before sampling, whereas Sabiston and his co-workers fail to describe their method of mucosal surface preparation. In fact, in a review in 1977, they point out the fact that no control was maintained over adequate mucosal surface preparation prior to sampling (Sabiston

and Grigsby, 1977) an obvious defect in that study.

b. Specimen Transport and Culture Techniques

Most studies do not describe the method used in transporting the specimen from the patient to the laboratory. Sabiston and his co-workers transported their specimens in the same syringe used for the aspiration immediately after obtaining the sample. Some more complex methods of transportation have been utilized, these include the anaerobic clinic cart (Fulghum, 1971) and the anaerobic specimen transport device (Wilkin and Jimenez-Ulata, 1975). In a recent study aimed at evaluating the various methods of sample transport and subsequent cultivation of bacterial specimens from infected dental root canals, it was shown that the use of these transport methods did little to actually improve the recovery of microorganisms and, in fact, tended to lead to a higher risk of specimen contamination (Carlsson and Sundqvist, 1980).

The majority of early investigation on dental periapical abscesses actually failed to utilize adequate anaerobic culture techniques (Alin and Ågren, 1954; Melville and Birch, 1967; Goldberg, 1970; Turner, Moore, and Shaw, 1975). In later studies, techniques using anaerobic jars with or without a roll tube technique have been used (Feldmann and Larje; Sabiston and Gold, 1974; Sabiston, Grigsby, and Segerstrom, 1976). Although this is an improvement on previous methods, they still have their drawbacks, for example, streaking of culture plates on bench tops, and the fact that reduction of the atmosphere within the jar requires some

hours and thereby exposes the specimen to ambient oxygen for substantial periods of time (Sabiston and Grigsby, 1977). In addition, picking and transfer of colonies after primary culturing are usually accomplished with some exposure to the ambient environment, a process which may further reduce the chances for recovery of some highly oxygen-sensitive anaerobic bacteria. The use of an anaerobic chamber is currently considered the best technique available for recovery of stringent anaerobes since specimens and cultures can be protected from oxygen at every stage of the procedure (Sabiston and Grigsby, 1977). To date, no studies on the microbiology of dental periapical abscesses have been reported which have utilized such a culturing technique.

All previous studies utilized different culture media for primary isolation which may well explain the variance in the previously published data. Blood agar plates were almost universally used, but their composition unfortunately varied widely. Examples of non-selective media recommended for growth of oral bacteria include MM105 agar (Syed and Loesche, 1973) and Trypticase Soy with 5% sheep blood agar supplemented with Vitiman K and hemin (Dowell and Hawkins, 1974). Good results were obtained with the various types of blood agar plates provided they were stored under anaerobic conditions prior to use. In addition to the use of a non-selective medium, the use of selective media has also been recommended where mixed bacterial populations are suspected (Dowell and Hawkins, 1974) and this was not done extensively in many early studies.

TABLE I

BACTERIOLOGIC STUDIES OF DENTAL ABSCESSES

REFERENCE	DESCRIPTION OF LESION	# OF CASES	# WITH ANAEROBES	ANAEROBES ISOLATED	FACULTATIVE AND ANAEROBES ISOLATED
Head and Ross, 1919	Apical Abscess	100	90 (?)	Gm. negative cocco bacilli (90) Bacillus bifidus (10)	Streptococci, mostly Viridans strep.
Davis and Moore-head, 1931	Chronic Alveolar Abscess	20	20	Fusiforms (20) Spirochetes (16)	Viridans strep. (20)
Bulleid, 1931	Acute Alveolar Abscess	16	3	Gram negative bacilli	Yes, not specified
Alin and Ågren, 1954	Acute Alveolar Abscess	27 (NG) 8	0		Strep. viridans (19)
Feldmann and Larje, 1966	Submucous Abscess	73 (NG) 9	27	Anaerobic strep. (12) Veillovella sp. (7) Leptotrichia sp. (3) Fusobacteria sp. (4) Bacteroides sp. (1)	Strep. Viridans (47) Strep. faecalis (4) Staph. albus (2) Diphtheroides (14) Neisseria (7)
Sabiston and Gold, 1974	Acute Alveolar Abscess	8	7	Fuso. nucleatum (7) Fusobacterium sp. (1) Bacteroides sp. (4) Peptostrep. sp. (2) Lactobacillus sp. (2) Actinomyces sp. (1)	Facult. strep. (6) Gm.+ facult. rod (1) Staph. epidermis (1)
Turner, Moore, and Shaw, 1975	Acute soft tissue abscess	66	7 (?)	Actinomyces sp. (2) Lactobacillus sp. (1) Peptostrep. sp. (2) Gm. -anaerobic rod (1)	Strep. viridans (42) Staph. epidermis (9) Staph. aureus (4)
Sabiston, Grigsby, and Segerstrom, 1976	Pyogenic infection of dental origin	65 (NG) 7	48	Gm. positive rods (24) Gm. negative rods (40) Gm. positive rods (28) Gm. negative rods (10)	Gm. + rods (10) Gm. - rods (3) Gm. + rods (42) Gm. - rods (5)

OBJECTIVES OF THIS STUDY

Thus, the nature of the microbiota of dental periapical abscesses has not been definitely established. Our hypothesis is that the predominant bacterial types in dental periapical abscesses are similar to those found in necrotic dental pulps. Necrotic dental pulps have been previously shown to be supportive of primarily anaerobic and facultatively anaerobic bacteria (Kantz and Henry, 1974; Whittgow and Sabiston, 1975; Sundqvist, 1976). Therefore, any study of the microbiology of dental periapical abscesses should thus utilize techniques which would permit the recovery of such microorganisms. The specific objective of this study was, therefore, to isolate and identify the most frequent types of microorganisms found in such lesions *in situ*.

It would further appear important to discriminate between the possible presence of microorganisms in such lesions as casually related to the inflammatory tissue destructive process or as incidental to the causation of the lesion due to other factors.

MATERIALS AND METHODS

SUBJECTS

Ten patients, aged between 20 and 65 years were studied from persons who presented at the University of Connecticut Health Center Dental Clinics with dental abscesses of suspected pulpal origin. The confirmatory diagnosis for these abscesses was made on the basis of symptoms, clinical examination, pulp sensitivity testing, and radiographic data. The patient's health questionnaire was reviewed to ensure that they did not have any of the following medically compromising conditions: a previous history of rheumatic fever, heart murmur or other heart ailments, diabetes or other systemic medically compromising conditions, or antibiotic therapy within two months of presenting at the dental clinic. In addition, if the abscess was associated with teeth whose roots were in close relationship with the maxillary sinus, or with teeth with a deep proable periodontal pocket, or with teeth in which the abscess was accompanied by a sinus drainable tract, then such patients were also excluded from this study.

From those patients who met the above stated criteria, abscess exudates were obtained for microbiological examination after the patient had consented to participate in the study. A copy of the consent form is included on the following page.

CONSENT FORM

I understand that I am being asked to participate in an experiment conducted by Dr. Oguntebi to determine if the abscess at the end of the root of my tooth is due to an infection. If it is due to an infection, Dr. Oguntebi will attempt to find out what kind of bacteria are causing it.

I understand that Dr. Oguntebi will sterilize the gum over my sore tooth and will give me one or more injections of local anesthetic to try to numb my tooth. I understand he has told me that while in most cases this completely numbs the area, in some cases it fails to completely do so because it is inflamed.

Dr. Oguntebi will insert a needle to the abscess near the root of my tooth and draw up some pus which he will examine for the presence of bacteria. This process may indeed relieve some of my discomfort by releasing some pressure from the area. If he feels that more drainage is required, he will insert the kind of sterile rubber drain standardly used in Endodontic (root canal) practice. He will then return me to my doctor who will perform standard procedures of opening my tooth to allow it to drain, start the healing process, and begin the treatment process.

I understand that I cannot expect to benefit from the experimental portion of this procedure (the determination of whether or not the abscess is infected with bacteria and what bacteria are present). The other procedures are standard ones generally used in Endodontics (root canal) treatment for abscessed teeth.

I am free to refuse to participate in this experiment, and I understand that this clinic will still take good care of me. I understand that the risks to me of this procedure are no greater than the risk ordinarily encountered in root canal treatment.

It is not the policy of the University of Connecticut which is funding the research project in which you are participating to compensate or provide treatment for human subjects in the event the research results in physical injury. The University of Connecticut Health Center/John Dempsey Hospital in fulfilling its public responsibility provides professional liability coverage for any injury in the event such injury is caused by the fault of the University of Connecticut Health Center/John Dempsey Hospital, its employees or agents.

In the event you believe that you have suffered any physical injury as the result of the participation in the research program, please contact Mrs. Jane Johnson, phone number: 674-2142, who can review the matter with you, and provide further information as to how to proceed.

I, the undersigned, have understood the above explanations and give consent to my voluntary participation in Dr. Oguntebi's research project.

_____	_____
Date	Signature of Subject
_____	_____
Location	Signature of Parent/Guardian
_____	_____
Witnessed by	Date

COLLECTION OF ABSCESS CONTENTS

Prior to the collection of the abscess contents the oral mucosa overlying the abscess was scrubbed with a 5% tincture of iodine. The area was subsequetnly anesthetized using an appropriate block injection of Lidocaine 2% (w/v) with epinephrin (1:100,000). A sterile 18-guage needle, fitted to a 5 cm^3 disposable plastic syringe, was passed to the focus of the abscess and the contents withdrawn. Following its withdrawl, the needle was sealed by inserting it into a sterile rubber block. The whole assembly was placed into a brewer-type jar (B.B.L. Cockeysville, Md.) and transported within 10 minutes to an anaerobic chamber (Coy Manufacturing, Michigan), maintained at 37^{o}C with a constant atmosphere of 85% N_2, 10% H_2, and 5% CO_2.

SAMPLE HANDLING

The contents of the syringe were expelled into a culture tube containing prereduced Ringer's solution supplemented with cysteine (4.5 mg/ml) and sodium metaphosphate (5 mg/ml). The suspension was gently agitated to effect disruption of bacterial clumps. Aliquots were removed and the morphology of the cells types and such traits as motility determined microscopically utilizing both phase contrast and Normarski optics. A portion was also gram stained. The remainder of the sample was serially diluted in the Ringer's solution and 0.02 ml of several dilutions plated onto each of the following media: Trypticase soy agar containing 5% (v/v) sheep red blood cells (Manganiello, et. al., 1977), Mitis salivarius agar (Man-

ganiello, et. al., 1977), an Actinomyces selective (CNAC-20) agar (Ellen and Bolcerzak-Raczkowski, 1975), an Fusobacterium selective medium (Mandel, Walter, and Socransky, 1979), and an Eikenella corrodens selective medium (Slee and Tanzer, 1978). All solid culture media were used as poured plates which were stored in the anaerobic chamber for at least 24 hours before used. Plates were incubated under either anaerobic, microaerophilic, or aerobic atmospheres for up to 7 days at 37°C. Representative colonies were selected from all media and pure cultures were obtained for further species characterization. All cultures were grown and subsequently maintained in either Todd-Hewitt or Trypticase soy broths supplemented with potassium nitrate (2 mg/ml) and hemin (5 mg/ml). The microorganisms were identified by examination of their colonial morphologies, growth on selective and/or differential media, Gram reaction, nutritional requirements, carbohydrate fermentation abilities, and other biochemical tests such as catalase activity, nitrate reduction, indole test, esculin, starch, and gelatin hydrolysis, amino acid decarboxylase activities, and urease activity. All microorganisms isolated were tested for glucose fermentation abilities and acid end product analysis. In addition, all strains of Actinomyces and Streptococci were tested for their abilities to utilize the following carbohydrates: lactose, rhamnose, mannitol, maltose, sucrose, raffinose, xylose, trehalose, arabinose, cellobiose, salicin, sorbitol, and mannose.

Reference strains for the above stated biochemical tests

were obtained from Dr. Kenneth Kornman of the Department of Periodontics, University of Connecticut Health Center and also from Dr. Sigmund Socransky of Forsyth Dental Center, Boston, Massachusetts.

RESULTS

Ten patients meeting the stated selection criteria and with diagnostically confirmed dental periapical abscesses were studied. A total of 25 bacterial strains were isolated with 10 isolates, being Gram-positive faculative anaerobic cocci, 3 Gram-positive anaerobic cocci, 9 Gram-negative anaerobic rods, and 3 Gram-positive anaerobic cocci. Bright-field microscopic examination of the samples failed to reveal the presence of Gram-negative cocci and, in addition, phase contrast Normarski or darkfield microscopy failed to reveal the presence of spirochaetes or morphologic forms not detected in direct culture. Detailed results of the identification of pure culture isolates from the dental periapical abscesses of the ten patients are presented in Tables II-X.

The actual distribution of those isolated strains is shown in Table XI. It may be noted that unlike the periodontal abscess or necrotic pulp tissue, the periapical abscess does not appear to support a plethora of microbial species because at no time were they found to harbor more than four bacterial species and six of the abscesses harbored only two strains. In seven of the cases, both facultative anaerobes and obligate anaerobes were found in the exudates and only in two cases were only facultative anaerobes found. Similarly, only one abscess contained just an obligately

anaerobic microflora. This patient, it should be noted, had a partially filled root canal. However, there was no readily discernible correlation between the state of health of the tooth associated with the abscess and the nature of the recoverable microflora. The predominant microorganisms isolated from dental periapical abscesses in this study were Fusobacterium nucleatum and Streptococcus mitis.

One strain of Staph. epidermis was recovered and was considered a possible skin contaminant during the sample collection and handling.

KEY TO TABLES II-XI, XIII-XV

COLONIAL MORPHOLOGY ON BLOOD AGAR (BA)

Color	Elevation	Margin
1. Pigmented	1. Depressed	1. Entire
2. White	2. Flat	2. Undulate
3. Yellow	3. Convex	3. Erose
4. Clear	4. Pulvinate	4. Filamentous
5. Iridescent	5. Umbonate	5. Curled
		6. Star shaped

(α) = α-haemolytic
(β) = β-haemolytic

BA = Blood Agar
BAB = basal Anaerobic broth

ACID END PRODUCT ANALYSIS BY GAS LIQUID CHROMATOGRAPHY (GLC)

F = Formic acid
A = Acetic acid
Pr = Propionic acid
IB = Isobutyric acid
B = Butyric acid
IV = Isovaleric acid
IC = Isocaproic
C = Caproic acid
H = Heptanic acid
Py = Pyruvic acid
L = Lactic acid
S = Succinic acid
PA = Phenylacetic acid
NT = Not Tested

AMINO ACID DECARBOXYLASE

Lys. = Lysine

Orn. = Ornithine

TABLE II

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES	(SEM)
	1	2
Aerobic Growth	-	+
Anaerobic Growth	+	+
Colonial Morphology on BA	2, 3, 1 (x)	2, 3, 1
Acid end products by GLC	A, Pr, B, IC, S	A, IB, B, IC, L, S
Gram Reaction	Gm+ cocci, chains	Gm+ cocci, chains
Terminal pH in BAB	5.2	6.8
Catalase	-	±
Nitrate Reduction	-	±
Indole	-	-
Esculin hydrolysis	-	-
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-
Acid production from:		
glucose	+	+
lactose	+	-
rhamnose	±	-
mannitol	±	-
maltose	+	+
sucrose	+	+
raffinose	-	-
xylose	+	-
trehalose	+	+
arabinose	+	-
cellobiose	+	-
salicin	+	+
sorbitol	+	-
mannose	+	-
IDENTIFICATION	Peptostrep. anaerobius	Strep. mitis

TABLE III

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (MDI)			
	1	2	3	4
Aerobic Growth	-	+	-	-
Anaerobic Growth	+	+	+	+
Colonial Morphology on BA	1, 4, 1	4, 3, 1 (x)	5, 2, 1	4, 3, 1
Acid end products by GLC	A, Pr, S	A, L, S	A, Pr, S	A, B, IC, S
Gram Reaction	Gm- cocci-bacilli	Gm+ cocci chains	Gm+ rods	Gm+ cocci chains
Terminal pH in BAB	5.2	6.9	6.3	5.3
Catalase	-	-	-	-
Nitrate Reduction	-	-	-	-
Indole	+	±	+	+
Esculin hydrolysis	-	-	-	-
Starch hydrolysis	-	-	-	-
Gelatin liquefaction	+	-	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-	-	-
Acid production from:				
glucose	NT	+		±
lactose	NT	-		+
rhamnose	NT	-		±
mannitol	NT	-		±
maltose	NT	+		+
sucrose	NT	+		+
raffinose	NT	±		±
xylose	NT	-		+
trehalose	NT	±		+
arabinose	NT	-		+
cellobiose	NT	-		+
salicin	NT	+		+
sorbitol	NT	-		±
mannose	NT	-		-
IDENTIFICATION	B. mel. (int.)	Strep. mitis	F. nucleatum	Peptostrep anaerobius

TABLE IV

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (MCH)		
	1	2	3
Aerobic Growth	-	+	+
Anaerobic Growth	+	+	+
Colonial Morphology on BA	5, 3, 2	2, 3, 1 (x)	3, 3, 1
Acid end products by GLC	A, Pr, Ib, B, S	A, B	F, A, L, S
Gram Reaction	Gm- filamentous rods	Gm+ cocci, chains	Gm+ rods
Terminal pH in BAB	6.0	7.1	5.9
Catalase	-	-	-
Nitrate Reduction	-	-	+
Indole	+	+	-
Esculin hydrolysis	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	-	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-	-
Acid production from:			
glucose	-	-	-
lactose	NT	-	-
rhamnose	NT	-	-
mannitol	NT	-	-
maltose	NT	+	+
sucrose	NT	+	+
raffinose	NT	-	-
xylose	NT	-	-
trehalose	NT	-	+
arabinose	NT	-	-
cellobiose	NT	-	-
salicin	NT	+	+
sorbitol	NT	-	+
mannose	NT	-	-
IDENTIFICATION	Fusobacterium nucleatum	Strep. mitis	Actino. Viscosus

TABLE V

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (GS)	
	1	2
Aerobic Growth	-	-
Anaerobic Growth	+	+
Colonial Morphology on BA	5, 3, 2	2, 3, 1
Acid end products by GLC	A, Pr, B, S	A, B, IC, S
Gram Reaction	Gm- filamentous rods	Gm+ cocci, chains
Terminal pH in BAB	6.2	5.3
Catalase	-	-
Nitrate Reduction	-	-
Indole	+	-
Esculin hydrolysis	-	-
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-
Acid production from:		
glucose	-	+
lactose	NT	-
rhamnose	NT	-
mannitol	NT	-
maltose	NT	-
sucrose	NT	-
raffinose	NT	-
xylose	NT	-
trehalose	NT	-
arabinose	NT	-
cellobiose	NT	-
salicin	NT	-
sorbitol	NT	-
mannose	NT	-
IDENTIFICATION	Fuso. nucleatum	Peptostrep. micros.

TABLE VI

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (JS)	
	1	2
Aerobic Growth	+	+
Anaerobic Growth	+	+
Colonial Morphology on BA	3, 3, 1	4, 3, 1
Acid end products by GLC	F, A, L, S	A, L, S
Gram Reaction	Gm+ rods	Gm+ cocci chains
Terminal pH in BAB	5.7	5.6
Catalase	-	-
Nitrate Reduction	+	-
Indole	-	-
Esculin hydrolysis	+	-
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-
Acid production from:		
glucose	+	+
lactose	-	+
rhamnose	-	+
mannitol	-	+
maltose	+ -	+
sucrose	+	+
raffinose	-	-
xylose	-	-
trehalose	+	+
arabinose	-	-
cellobiose	-	+
salicin	+	+
sorbitol	+	+
mannose	-	+
IDENTIFICATION	Actino. viscosus	Strept. faecalis

TABLE VII

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (GM)		
	1	2	3
Aerobic Growth	-	-	+
Anaerobic Growth	+	+	+
Colonial Morphology on BA	1, 3, 1	5, 3, 2	4, 3, 1
Acid end products by GLC	A, Pr, B, S	A, Pr, S	A, L, S
Gram Reaction	Gm- coccobacilli	Gm- filamentous rods	Gm+ cocci, chains
Terminal pH in BAB	5.3	6.4	5.3
Catalase	-	-	-
Nitrate Reduction	-	-	-
Indole	+	+	-
Esculin hydrolysis	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	+	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-	-
Acid production from:			
glucose	+	-	+
lactose	NT	NT	-
rhamnose	NT	NT	-
mannitol	NT	NT	-
maltose	NT	NT	+
sucrose	NT	NT	+
raffinose	NT	NT	-
xylose	NT	NT	-
trehalose	NT	NT	-
arabinose	NT	NT	-
cellobiose	NT	NT	-
salicin	NT	NT	-
sorbitol	NT	NT	-
mannose	NT	NT	-
IDENTIFICATION	B. mel. (int)	Fuso. necleatum	Strep. mitis

TABLE VIII

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (BM)	
	1	2
Aerobic Growth	+	-
Anaerobic Growth	+	+
Colonial Morphology on BA	2, 3, 1	5, 3, 2
Acid end products by GLC	A, L, S	A, Pr, S
Gram Reaction	Gm+ cocci chains	Gm- rods
Terminal pH in BAB	5.6	6.8
Catalase	-	-
Nitrate Reduction	-	-
Indole	-	+
Esculin hydrolysis	-	-
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Amino acid decarboxylase $\frac{\text{Lys.}}{\text{Orn.}}$	-	-
Acid production from:		
glucose	+	-
lactose	+	NT
rhamnose	+	NT
mannitol	+	NT
maltose	+	NT
sucrose	+	NT
raffinose	-	NT
xylose	±	NT
trehalose	+	NT
arabinose	-	NT
cellobiose	+	NT
salicin	+	NT
sorbitol	+	NT
mannose	+	NT
IDENTIFICATION	Strep. faecalis	Fuso. nucleatum

TABLE IX

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (SIM)		
	1	2	3
Aerobic Growth	+	+	+
Anaerobic Growth	+	+	+
Colonial Morphology on BA	2, 3, 1	4, 3, 1	3, 3, 1
Acid end products by GLC	A, L, S	A, L, S	F, A, L, S
Gram Reaction	Gm+ cocci chains	Gm+ cocci chains	Gm+ rods
Terminal pH in BAB	5.4	6.2	5.6
Catalase	-	-	+
Nitrate Reduction	-	-	±
Indole	±	±	-
Esculin hydrolysis	-	-	+
Starch hydrolysis	-	-	-
Gelatin liquefaction	-	-	-
Amino acid decarboxylase Lys. / Orn.	-	-	-
Acid production from:			
glucose	+	+	+
lactose	+	+	-
rhamnose	±	±	-
mannitol	+	+	-
maltose	+	+	±
sucrose	+	+	±
raffinose	-	-	-
xylose	±	±	-
trehalose	+	+	+
arabinose	-	-	-
cellobiose	+	+	-
salicin	+	+	+
sorbitol	+	+	+
mannose	+	+	-
IDENTIFICATION	Strep. faecalis	Strep. faecalis	Actino. viscosus

TABLE X

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (CM)		
	1	2	3
Aerobic Growth	+	-	+
Anaerobic Growth	+	+	+
Colonial Morphology on BA	2, 3, 1 (x)	5, 3, 2	3, 3, 1
Acid end products by GLC	A, L, S	A, Pr, B, S	NT
Gram Reaction	Gm+ cocci chains	Gm- filamentous rods	Gm+ cocci clumps
Terminal pH in BAB	7.1	7.6	7.3
Catalase	+	-	+
Nitrate Reduction	-	-	+
Indole	-	+	-
Esculin hydrolysis	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	-	-	-
Amino acid decarboxylase $\frac{Lys.}{Orn.}$	-	-	-
Acid production from:			
glucose	+	NT	NT
lactose	+	NT	NT
rhamnose	-	NT	NT
mannitol	-	NT	NT
maltose	+	NT	NT
sucrose	+	NT	NT
raffinose	-	NT	NT
xylose	-	NT	NT
trehalose	-	NT	NT
arabinose	-	NT	NT
cellobiose	-	NT	NT
salicin	-	NT	NT
sorbitol	-	NT	NT
mannose	-	NT	NT
IDENTIFICATION	Strep. mitis	Fuso. nucleatum	Staph. epidermis

TABLE XI

CHARACTERIZATION OF PURE CULTURE ISOLATES FROM TEN DENTAL PERIAPICAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (VFA)	
	1	2
Aerobic Growth	-	+
Anaerobic Growth	+	+
Colonial Morphology on BA	5, 3, 1	2, 3, 1
Acid end products by GLC	A, Pr, B, S	A, L, S
Gram Reaction	Gm- filamentous rods	Gm+ cocci chains
Terminal pH in BAB	7.5	6.7
Catalase	-	-
Nitrate Reduction	-	-
Indole	+	-
Esculin hydrolysis	-	-
Starch hydrolysis	-	-
Gelatin liquefaction	-	-
Amino acid decarboxylase Lys. / Orn.	-	-
Acid production from:		
glucose	-	+
lactose	NT	-
rhamnose	NT	-
mannitol	NT	-
maltose	NT	±
sucrose	NT	+
raffinose	NT	-
xylose	NT	-
trehalose	NT	-
arabinose	NT	-
cellobiose	NT	-
salicin	NT	-
sorbitol	NT	-
mannose	NT	-
IDENTIFICATION	Fuso. nucleatum	Strep. mitis

TABLE XII

THE MICROFLORA ASSOCIATED WITH
DENTAL PERIAPICAL ABSCESSES

SUBJ.	SEX & AGE	TOOTH DESIGNATION* & DENTAL HISTORY	NO. OF BACTERIAL STRAINS ISOLATED	MICROFLORA
1	M/65	46, Temporary Crown	2	Streptococcus mitis Peptostreptococcus anaerobius
2	F/38	25, Carious exposure of the pulp	4	Streptococcus mitis Fusobacterium nucleatum Bacteroides melaninogenicus s. s. intermedius Peptostreptococcus anaerobius
3	F/29	46, Defective amalgam filling	3	Streptococcus mitis Actinomyces viscosus Fusobacterium nucleatum
4	M/35	46, Partial root-filling	2	Fusobacterium nucleatum Peptostreptococcus micros.
5	M/26	26, Defective amalgam filling	3	Streptococcus mitis Fusobacterium nucleatum Bacteroides melaninogenicus s. s. intermedius
6	M/29	37, Carious exposure of the pulp	2	Streptococcus faecalis Actinomyces viscosus
7	F/24	24, Defective amalgam filling	2	Streptococcus faecalis Fusobacterium nucleatum
8	M/24	36, Carious exposure of the pulp	2	Streptococcus faecalis Antinomyces viscosus
9	F/20	11, History of trauma Intact Crown	3	Streptococcus mitis Staphylococcus epidermis Fusobacterium nucleatum
10	M/32	11 & 21, History of trauma, Intact Crowns	2	Streptococcus mitis Fusobacterium nucleatum

*FDI Designation

DISCUSSION

A dental periapical abscess is a localized collection of pus in the alveolar bone at the root apex of a tooth. The microbiological methodologies hitherto employed in the examination of such sites have simply involved crude culturing with uncontrolled exposure to the ambient environment prior to enumeration and subsequent characterization (Alin and Agren, 1954; Melville and Birch, 1967; Goldberg, 1970; Turner et. al., 1975). Furthermore, cultivation has invariably occured in non-reduced atmospheres and generally with complex growth media, which tends to select for those species which grow with a short growth lag and generation time. Obviously, the use of what may be construed as less-than-ideal conditions would not permit the recovery of various anaerobic and nutritionally fastidious microorganisms if such were resident in the particular ecological niche. In this study, abscess exudates were transported directly under a reduced atmosphere to an anaerobic environment where subsequent microbiological examinations occured within a short time of the initial sample taking. The recent studies of Carlsson and Sundqvist (1980) have clearly shown that such minimal handling conditions are certainly prerequisite for increasing the recovery of bacteria from root canals and at the same time reducing the risk of specimen contamination.

The microbiological analysis of the exudates from these dental abscesses indicated that such lesions have a somewhat specific microflora. Such is apparently in direct contrast with that normally observed for the periodontal abscesses which appear to be supportive of a very heterogenous bacterial population (Newmann and Sims, 1979). Similarly, the microflora observed in this study was also more limited than that previously described in an earlier study by Sabiston and Gold, who reported a much higher recovery of Bacteroides species. Caution should be taken in the interpretation of their findings, however, as they sampled abscesses of both periodontal and pulpal origin but unfortunately failed to make a clear distinction between them. Such a distinction appears crucial especially in view of the studies on the microflora associated with periodontal abscess recently reported by Newmann and Sims in which they demonstrated that periodontal abscesses are clearly supportive of a very heterogeneous bacterial population. It would thus appear possible that the heterogeneous bacterial population and the higher recovery of Bacteroides species reported by Sabiston and his co-workers were more reflective of bacterial recoveries from periodontal rather than pulpal sources.

In the present study, F. nucleatum and S. mitis were the most predominant microorganisms isolated. Generally, it was also observed that an obligate anaerobe was accompanied by a facultative anaerobic microorganism although in two of the cases, a mixed infection occurred with facultative organisms and also in one instance, two anaerobes were found.

However, all abscesses may be considered to be representative of a mixed microbial infection of microorganisms that occur as part of the normal oral flora. Similar mixed infections have also been proposed to be involved in the pathogenesis of various periodontal diseases (MacDonald, Socransky, and Gibbons, 1963). Recent studies (Sundqvist et. al., 1979) pertaining to the capacity of bacteria isolated from necrotic pulp tissue to induce transmissible purulent infections have demonstrated in the guinea pig skin abscess model system used, the necessity of bacterial combinations to achieve pathogenicity. The findings of this study tends to support this concept with respect to the pathogenesis of dental periapical abscesses.

SUMMARY AND CONCLUSIONS

1. The predominant microflora associated with human dental periapical abscesses following pulpal necrosis was investigated in a pilot study involving 10 patients from the Dental School of the University of Connecticut Health Center. Samples of pus were obtained by permucosal puncture and subjected to analysis using current anaerobic as well as aerobic cultivation techniques. This study indicates that the bacterial flora of exudates from clinically diagnosed dental periapical abscesses and necrotic dental pulps, was rather specific. The largest number of bacterial species recovered from an abscess was 4 and the least was 2. There were no pure culture infections and no abscesses were sterile.
2. Altogether, 25 bacterial strains were isolated and comprised the following morphologic and physiologic types. Ten Gram-positive facultative anaerobic cocci, 3 Gram-positive anaerobic cocci, 3 Gram-positive facultative anaerobic rods, and 9 Gram-negative anaerobic rods.
3. No Gram-negative cocci or spirochetes were cultured or observed in stained or wet mount preparations viewed by brightfield, phase contrast, or Normarski optics.

4. The microorganisms isolated most frequently were determined to be Fusobacterium nucleatum and Streptococcus mitis, and they occurred in six of the ten cases that were examined.

5. Further studies are required involving a larger number of cases to confirm the findings of this study. If the findings of this study are confirmed, it would be interesting to test the pathogenicity of these two major isolates in an animal model system. The development of a rapid technique of identification of these microorganisms, such as immunofluorescence could also be a good adjunct to the rapid diagnosis and treatment of such lesions.

APPENDIX

PILOT EXPERIMENT A

Objective: To test the effectiveness of the methods proposed in the protocol and described under Materials and Methods in the main body of this thesis for recovery of microorganisms from samples of dental periapical abscesses and a sample of periodontal abscess.

Materials and Methods: Four patients aged 28 to 46 years meeting criteria outlined in protocol were studied among patients who presented with dental abscesses. Two of the patients were sampled at the University of Connecticut Health Center (UCHC) dental clinics, one at the Burgdorf Health Center dental clinic where a large number of cases have been reported, and one in a private dental office. Of the two cases sampled at the UCHC dental clinics, one was a periapical abscess associated with pulp necrosis while the other was a periodontal abscess that was associated with a deep periodontal pocket on a tooth with a vital pulp. The two cases sampled at the Burgdorf Health Center dental clinic and in the private dental officer were both periapical abscesses associated with pulp necrosis. Sample collection and handling were performed as outlined in the body of this thesis under Materials and Methods.

These different samples were obtained in order to:

1. Test the recovery of microorganisms from the various sources and assess the effect of transport time on the recovery of the microorganisms. The samples collected from the University of Connecticut Health Center dental clinics were transported to the anaerobic chamber within 10 minutes of collection, whereas those obtained at the Burgdorf Health Center dental clinic and the private dental office were introduced into the anaerobic chamber after 45 minutes and 30 minutes, respectively.

2. A sample of a periodontal abscess was taken to test how well our methodology was able to recover the types of microorganisms that have been described in such sites by previous investigations.

3. A detailed analysis of these samples was carried out to identify the types of microorganisms present in such sites and act as a guide in the selection of culture media and methods of their characterization of isolates such as biomechanical tests.

Results: From these abscess samples, a total of 12 bacterial strains were isolated belonging to a variety of species. Eight of the 12 strains were isolated from the one sample of periodontal abscess. The sample taken from the private dental office yielded one isolate and no isolate was recovered from the sample taken from the Burgdorf Health Center dental clinic. Characterization and identification of pure culture isolates from the four patients are shown in Tables XII-XIV. The actual distribution of the strains isolated is shown in Table XV. Of the 12 iso-

lates, 3 were Gram-positive facultative cocci, 6 were Gram-negative anaerobic rods, 1 was a Gram-positive cocci, and 1 was a Gram-negative anaerobic cocci.

Conclusions: The results indicate that dental abscesses may support a large variety of bacterial types but possibly a predominance of anaerobes. It would appear important, therefore, to include techniques optimal for their isolation. It would also appear important to include many methods of characterization in addition to a battery of biochemical tests to ensure precision in identification of the micro-organisms isolated.

Samples obtained from sources outside the University of Connecticut Health Center gave a poor recovery of micro-organisms. This would appear to be due to the prolonged time interval between sample collection and start of analysis in the anaerobic chamber. Sampling would, therefore, be restricted to cases within the University of Connecticut Health Center dental clinics. If more samples are needed, these other sources will be explored using improved methodologies such as the use of a transport medium.

PILOT EXPERIMENT B

Objective: To test the reliability of the different culture media selected for the study.

Materials and Methods: 0.02 ml of log phase cultures of the 12 bacterial strains isolated from Pilot Experiment A as well as 2 strains of Bacteroides melaninogenicus, one strain of Eikenella corrodens and one strain of Bacteroides

corrodens were plated onto the selective and non-selective media described in the protocol under sample handling. Replicate plates were incubated under anaerobic (in the anaerobic chamber), microaerophilic (in candle jars) and aerobic atmospheres for up to 7 days at 37°C. Growth of the microorganism was checked and recorded daily.

Results: Growth of the microorganisms in the various culture media occurred as shown in Table XVI. Growth was recorded as + and no growth was recorded as -.

Conclusions: Trypticase soy agar containing 5% sheep red blood appear to be an effective basal medium to use for primary isolation of all the microorganisms isolated from the 4 dental abscesses sampled in Pilot Experiment A as well as for the growth of other bacterial types that have been isolated from other oral sources.

The various selective media chosen appear adequate for the growth of the microorganism for which it was originally designed. However, various strains of streptococci appear to grow well on most of these selective media. Thus, the various other methods of characterization proposed in the protocol would appear important in the final identification of isolates.

TABLE XIII

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM FOUR DENTAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (AK) PERIAPICAL ABSCESS		
	1	2	3
Aerobic Growth	-	-	+
Anaerobic Growth	+	+	+
Colonial Morphology on BA	1, 3, 1	5, 3, 2	2, 3, 1
Acid end products by GLC	A, B, IB	A, Pr, B, S	A, L, S
Gram Reaction	Gm- coccobacilli	Gm- filamentous rods	Gm+ cocci chains
Terminal pH in BAB	5.1	5.2	6.8
Catalase	+	-	-
Nitrate Reduction	-	-	-
Indole	+	+	-
Esculin hydrolysis	-	-	-
Starch hydrolysis	-	-	-
Gelatin liquefaction	+	-	-
Amino acid decarboxylase	-	-	-
IDENTIFICATION	B. asacch	F. nucleatum	Facult. strep.

TABLE XIV

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM FOUR DENTAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES (HE) PERIODONTAL ABSCESS							
	1	2	3	4	5	6	7	8
Aerobic Growth	-	-	-	-	+	+	-	-
Anaerobic Growth	+	+	+	+	+	+	+	+
Colonial Morphology on BA	1, 4, 1	1, 4, 1	5, 3, 1	2, 3, 1	2, 3, 1	2, 3, 1	4, 3, 2	2, 3, 1
Acid end product by GLC	A, B, IB	A, Pr, S	A, Pr, B, S	A, IC, S	A, L, S	F, A, L, S	A, Pr, B, L, S	A, P, B, S
Gram Reaction	Gm- cocco bacilli	Gm- rods	Gm- filament rods	Gm- cocci	Gm+ cocci chains	Gm+ rods	Gm+ rods	Gm+ cocci chain
Terminal pH in BAB	5.0	5.1	5.2	5.2	5.3	5.0	5.1	5.1
Catalase	+	-	-	+	-	+	-	-
Nitrate Reduction	-	-	-	+	-	+	+	-
Indole	+	+	+	-	-	-	-	-
Esculin hydrolysis	-	-	-	-	-	+	-	-
Starch hydrolysis	-	-	-	-	-	-	-	-
Gelatin liquifaction	+	+	-	-	-	-	-	-
Amino acid decarboxylase	-	-	-	-	-	-	-	-
IDENTIFICATION	B. asacch	B. mel	F. nuclea.	Veill. sp.	Facult. strep	Actino. sp.	Lep. buc.	Pepto-strep.

TABLE XV

CHARACTERIZATION OF PURE CULTURE ISOLATES
FROM FOUR DENTAL ABSCESS SAMPLES

CHARACTERISTICS	ISOLATES	PERIAPICAL ABSCESSES	
	(HC)	2	(BC)
Aerobic Growth	+		No
Anaerobic Growth	+		Growth
Colonial Morphology	2, 3, 1		
Acid end products by GLC	A, L, S		
Gram Reaction	Gm+ cocci chains		
Terminal pH in BAB	6.7		
Catalase	-		
Nitrate Reduction	-		
Indole	-		
Esculin hydrolysis	-		
Starch hydrolysis	-		
Gelatin	-		
Amino acid decarboxylase	-		
IDENTIFICATION	Facult. Strep		No Growth

TABLE XVI

THE MICROFLORA OF 4 CASES OF DENTAL ABSCESSES

SUBJ.	SOURCE	SEX & AGE	TOOTH DESIGNATION & DENTAL HISTORY	NO. OF BACTERIAL STRAINS ISOLATED	MICROFLORA
1	U. Conn. Health Ctr. Dental Clinic	M/28	15, Defective amalgam filling, facial cellulitis	3	Bacteroides melaninogenicus s. s. asaccharolyticus Fusobacterium nucleatum Strepto-coccus mitis
2	U. Conn. Health Ctr. Dental Clinic	F/36	16, Deep Periodontal pocket	8	Bacteroides asaccharolyticus Bacteroides melaninogenicus s. s. intermedius Fusobacterium nucleatum Veillonella sp. Streptococcus salivarius Actinomyces sp. Peptostrepto-coccus sp. Leptotrichia buccalis
3	Private Dental Office	M/46	33, Defective amalgam filling	1	Streptococcus faecalis
4	Burgdorf Health Ctr. Dental Clinic	M/29	16, Carious exposure of the pulp	0	----------

TABLE XVII

GROWTH OF ORAL MICROORGANISMS
IN DIFFERENT CULTURE MEDIA

	MS	TS5B	ECSM	CVE	CNAC-20
Streptococcus salivarius	+	+	-	-	+
Streptococcus faecalis	+	+	-	+	-
Streptococcus mitis	+	+	-	+	-
Peptostreptococcus	+	+	-	-	-
B. mel. asacch.	-	+	-	-	-
B. mel. inter.	-	+	-	-	-
Fusobacterium nucleatum	-	+	-	+	-
Veillonella sp.	-	+	-	-	-
Actinomyces sp.	-	+	-	-	+
Leptotrichia buccalis	+	+	-	-	-
Eikenella corrodens	-	+	+	-	-
B. mel. mel.	-	+	-	-	-
Bacteroides corrodens	-	+	-	-	-

MS = Mitis salivarius agar.
TS5B = Trypticase soy agar with 5% sheep red blood cells.
ECSM = Eikenella corrodens selective medium.
CVE = Fusobacterium selective medium.
CNAC-20 = Actinomyces selective medium.

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